

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
7 October 2004 (07.10.2004)

PCT

(10) International Publication Number
WO 2004/085619 A3

(51) International Patent Classification⁷: **C12N 15/82,**
A01H 5/00, 5/10

(21) International Application Number:
PCT/US2004/008807

(22) International Filing Date: 22 March 2004 (22.03.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/456,714 21 March 2003 (21.03.2003) US

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(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Euro-
pean (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR,
GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(88) Date of publication of the international search report:
25 November 2004

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: **EXPRESSION OF DESIRABLE PROTEINS IN PLANT ROOTS AND CELL CULTURES**

(57) Abstract: A new expression cassette for directing heterologous protein expression in plant roots has at least two portions, nucleotides encoding MsPRP2 promoter or a fragment thereof, said promoter or fragment comprising a portion of SEQ ID NO: 1; and nucleotides comprising a gene for a heterologous protein, operably linked to the MsPRP2 nucleotides. An expression cassette capable of directing heterologous protein expression in plant roots, comprising a) nucleotides encoding a promoter of MsPRP 2 or a fragment thereof; b) optionally nucleotides encoding a ribosomal binding site; c) optionally nucleotides encoding a secretion signal; and d) nucleotides encoding a heterologous protein, said protein nucleotides being operably linked to the MsPRP2 promoter nucleotides. The expression cassette further includes nucleotides encoding transcription factor Alfin1; the Alfin1 nucleotides are operably linked to another promoter such that the other promoter causes the transcription factor Alfin1 to be overexpressed. Also disclosed are plants and plant cells transfected with the preceding expression cassette. Also disclosed are methods for making plant cell-bound and secreted proteins. Seeds are made from transformed plant cells.

WO 2004/085619 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/08807

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/82; A01H 5/00, 5/10

US CL : 435/70.1, 320.1, 419, 468; 536/24.1; 800/287, 298

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/70.1, 320.1, 419, 468; 536/24.1; 800/287, 298

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database GenBank on STIC, National Center for Biotechnology Information, (Bethesda, MD, USA), Accession No. AF028841, DEUTCH et al., gene sequence for Medicago sativa proline-rich cell wall protein, 09 January 1999.	1-9
Y	BASTOLA et al. Alfin1, a novel zinc finger protein in alfalfa roots that binds to promoter elements in the salt-inducible MsPRP2 gene. Plant Molecular Biology. 1998. Vol. 38, pages 1123-1135, especially pages 1123, 1131, 1132-1134.	1-9
Y	SINGE et al. Phytoremediation: an overview of metallic ion decontamination from soil. Appl. Microbiol. Biotechnol. 26 February 2003, Vol. 61, pages 405-412, especially pages 409-410.	9



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:		"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

09 August 2004 (09.08.2004)

Date of mailing of the international search report

21 SEP 2004

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/08807

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material



a sequence listing



table(s) related to the sequence listing

b. format of material



in written format



in computer readable form

c. time of filing/furnishing



contained in the international application as filed



filed together with the international application in computer readable form



furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/08807

Continuation of B. FIELDS SEARCHED Item 3:

STN (agricola, biosis, biotechno, caba, caplus, medline, uspatfull): inventor name, MsPRP2, root, promoter, bioremediation. STIC
sequence search for SEQ ID NO:1.